Recommended Method For Testing the Objective Rancidity Development in Fish Based on TBARS Formation

C. Robles-Martinez, E. Cervantes and P. J. Ke

October 1982

Canadian Technical Report of Fisheries and Aquatic Sciences No. 1089

Canadian Technical Report of Fisheries and Aquatic Sciences

These reports contain scientific and technical information that represents an important contribution to existing knowledge but which for some reason may not be appropriate for primary scientific (i.e. *Journal*) publication. Technical Reports are directed primarily towards a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of the Department of Fisheries and Oceans, namely, fisheries management, technology and development, ocean sciences, and aquatic environments relevant to Canada.

Technical Reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report will be abstracted in *Aquatic Sciences and Fisheries Abstracts* and will be indexed annually in the Department's index to scientific and technical publications.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service. Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and the Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Details on the availability of Technical Reports in hard copy may be obtained from the issuing establishment indicated on the front cover.

Rapport technique canadien des sciences halieutiques et aquatiques

Ces rapports contiennent des renseignements scientifiques et techniques qui constituent une contribution importante aux connaissances actuelles mais qui, pour une raison ou pour une autre, ne semblent pas appropriés pour la publication dans un journal scientifique. Il n'y a aucune restriction quant au sujet, de fait, la série reflète la vaste gamme des intérêts et des politiques du Ministère des Pêches et des Océans, notamment gestion des pêches, techniques et développement, sciences océaniques et environnements aquatiques, au Canada.

Les Rapports techniques peuvent être considérés comme des publications complètes. Le titre exact paraîtra au haut du résumé de chaque rapport, qui sera publié dans la revue *Aquatic Sciences and Fisheries Abstracts* et qui figurera dans l'index annuel des publications scientifiques et techniques du Ministère.

Les numéros 1-456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457-714, à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715-924 ont été publiés à titre de Rapports techniques du Service des pêches et de la mer, Ministère des Pêches et de l'Environnement. Le nom de la série a été modifié à partir du numéro 925.

La page couverture porte le nom de l'établissement auteur où l'on peut se procurer les rapports sous couverture cartonnée.

CANADIAN TECHNICAL REPORT OF
FISHERIES AND AQUATIC SCIENCES
NO. 1089

OCTOBER 1982

RECOMMENDED METHOD FOR TESTING THE OBJECTIVE RANCIDITY DEVELOPMENT IN FISH BASED ON TBARS FORMATION

BY

C. Robles-Martinez*, E. Cervantes* and P. J. Ke

*Visiting Technologists from Mexican Council of Science and Technology

Department of Fisheries and Oceans
Fisheries Development Branch
P.O. Box 550, Halifax Laboratories
Halifax, Nova Scotia
Canada B3J 2S7

CONTENTS

	Page
Abstract	iv
Resume	v
Introduction	1
Experimental	
A. Preparation of Reagents	1
B. Preparation of Standard Curve	2
C. Apparatus	2
D. Recommended Procedure	2
Results and Discussion	
Comparison of Absorption Spectra	3
Recovery From TEP Standard and Investigation on Interfering Substances	3
Application For Fish Quality Evaluation	3
References	4
Table 1 - Completion on the Separation of TBARS by the Distillation Method	7
Table 2 - Recovery of TEP Standard Added to Various Fish Samples by Using the Recommended Method	9
Table 3 - Various Interferences From Some Biocompounds in Fish Tissue on the Recommended TBARS Determination	11
Table 4 - TBARS Value in Various Fresh and Frozen Fish Fillets Determined by the Distillation-Photometric Method	13
Table 5 - Recommended Guidelines on the Rancidity Qualtiy Assessment For Fresh and Frozen Fish Using TBARS Values	15
Figure 1A - Combined Distillation Assembly For TBARS Analysis	17
Figure 1B - Distillation Assembly For TBARS Analysis	19
Figure 2 - Standard Curve For TBARS Determination at 538 nm Using TEP as the Standard	21
Figure 3 - Spectra of Various TBARS From Fish Tissue	23
Figure 4 - Absorption Spectra of Various TBA-Carbonyl Complex Formed by Using the Described Distillation-Photometric Operation	25
Figure 5 - TBARS Value Change For Various Fish Samples Kept at -2°C, -15°C and -30°C Respectively	27

ABSTRACT

C. Robles-Martinez, E. Cervantes and P. J. Ke. 1982. Recommended Method for Testing the Objective Rancidity Development in Fish Based on TBARS Formation. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1089, pages.

An improved method for determining the degree of rancidity in fish tissues has been developed. The rancidity indicating compounds in fish samples which are quantitatively reacted with 2-thiobarbituric acid are digested, then separated directly by a specific distillation and estimated by spectrophotometric measurement at 538 nm. The operational errors, the interferences and the recovery of volatile carbonyls for the described procedure have been investigated respectively. The recommended technique has been employed satisfactorily for various fish quality evaluations with the overall deviation of 7% and a detection limit of 0.2 nmoles of thiobarbituric acid-reactive substances (TBARS) per 10 grams of fish meats. The specific procedure of the above test has been described so that it can be applied to fresh and frozen fish products for various quality enhancement investigations.

RESUME

Nous avons mis au point une méthode améliorée de détermination du degré de rancidité des tissus de poisson. Les indicateurs de rancidité d'échantillons réagissant quantitativement à l'acide 2-thiobarbiturique sont digérés et séparés directement par distillation spécifique et ensuite estimés par mesure spectrophotométrique à 538 nm. Nous avons examiné séparément les erreurs

opérationnelles, les interférences et la récupération des carbonyls volatils dans cette méthode. Cette dernière a été utilisée avec succès dans diverses évaluations de la qualité du poisson. La déviation dans l'ensemble est de 7%, et la limite de détection de 0,2 nmole de substance réagissant à l'acide thiobarbiturique (TBARS) par 10 grammes de chair de poisson. La marche spécifique de ce test est décrite dans le but de l'appliquer à divers produits de poisson frais et congelés dans des recherches sur l'amélioration de la qualité.

INTRODUCTION

For decades, efforts have been made to determine spoilage of fatty foods by suitable chemical methods. Since 1944, it was observed (18) that animal tissues which had been incubated aerobically produce a colour with 2-thiobarbituric acid (TBA). Bernheim and co-workers (3) found this colour to be the result of a complex formed from oxidation products of unsaturated fatty compounds and 2-thiobarbituric acid.

As is well known, the primary products of lipid oxidation are hydroperoxides and these are readily decomposed to secondary reaction products, particularly carbonyl compounds (9, 10, 15, 28). Throughout the course of oxidation, thiobarbituric acid-reactive substances (TBARS) values of trienes, tetraenes, pentaenes and hexaenes have been found to vary linearly with diene conjugation and oxygen uptake (8). Meanwhile, monoenes and dienes may not produce thiobarbituric acid-reactive substances (TBARS) (30). In other words, malonaldehyde (MA) or TBARS are formed mainly from the oxidation of fatty acids having three or more double bonds (20, 22, 42). The other TBARS which accompany MA are not well identified (19), but they appear to be one stable precursor of MA, possibly vinyl ketons, 2,4-dienals and/or 2,4,7-decatrienals (13, 19, 31, 35). It is mostly accepted that the red pigment formed in the TBA reaction is the condensation product (in a heat-acid induced reaction) of 1 mol of MA and 2 mols of TBA (12, 27, 35, 42).

In the past years, various methods have been developed for performing the TBA test on food products (4, 23, 26, 32, 33, 34, 36, 39, 40). These methods can be classified under two categories:

(a) Extraction - An acid solution of TBA is added to the food product followed by heating in a bath water to obtain maximum colour development, then the pigment is extracted with a suitable solvent and measured spectrophotometrically;

(b) Distillation - The food product, under acid conditions, is distilled and the TBA solution is added to a portion of the distillate which is then heated and the colour is measured directly in a spectrophotometer.

As it can be seen, the two methods are similar in that both employ heating of the sample at a low pH. The distillation procedure offers several advantages over the extraction. It is more sensitive (40); better remotion of MA from interfering substances (39); less oxidation of lipids during the test itself; MA is obtained in a clear aqueous solution so that its reaction product with TBA does not need to be extracted with solvents (36); the relationship of the rancid odour to TBARS and other volatile compounds can be more readily studied in the clear distillates (36); the volatile constituents of the sample are distilled over, thus avoiding any reaction of the TBA with non-volatiles from the sample (26, 37).

The main disadvantage of the distillation is that two colours may be formed, a red one with maximum absorbance at 530-540 nm and a yellow one with maximum absorbance at 450 nm. The red colour is stable but the yellow is not. The yellow pigment has been deduced to be caused by some carbonyls which come from some decomposed products of hydroperoxides as a result of further oxidative decomposition (2) or due to impurities of reagents (42). Therefore, it was found that by heating at near the boiling point of water for less than an hour, the formation of this yellow pigment can be avoided (25). A new extraction method without heating was developed but is less sensitive than the distillation method (40). Also, there is a method based on the different absorbance wavelengths of MA in relation to the pH. This method is simpler, rapid and specific, but its sensitivity is only about 40% of the distillation method (20).

The 2-thiobarbituric acid reaction has been widely used to determine rancidity in seafoods (17, 23) and correlates reasonably well with taste panel data (15). Although modifications to the original method exist (39, 43), difficulties may still be encountered when dealing with certain fish due to interfering factors. For this reason, the TBA method of Tarladgis *et al* (41), involving a direct distillation of various TBARS from acidic media has been successfully improved. Thiobarbituric acid-reactive substances values, which can be measured spectrophotometrically, thereby provide an objective indicator for evaluating the rancidity development and the overall frozen quality in fish.

EXPERIMENTAL

A. Preparation of Reagents

2-Thiobarbituric acid (BDH), propyl gallate, disodium EDTA, anti-bumping granules (BDH), standard 1,1,3,3 - tetraethoxy propane (TEP, MW 220, K&K Laboratories), are recommended to be used in this TBARS analysis. Other chemicals used for various interference investigations are ACS grade.

(i) TBA Reagent

Add 1.44 gm 2-thiobarbituric acid and 50 ml distilled water into a 500-ml volumetric flask with vigorous stirring (magnetic stirrer). Glacial acetic acid is then added until flask is two-thirds full. The mixture is vigorously stirred for ten minutes or until the 2-thiobarbituric acid is almost completely dissolved. The flask is then filled to the mark with glacial acetic acid.

(ii) TEP Standard Solution

An amount of 0.22 gm of 1,1,3,3 - tetraethoxy propane (TEP) is accurately weighed into a 100-ml volumetric flask and diluted to volume with distilled water. Ten millilitres of this solution is pipetted into a one-litre volumetric flask and diluted to volume with distilled water to produce a 1×10^{-4} M stock solution. The solution is kept under refrigeration. A 1×10^{-5} M

working solution is then prepared by diluting 10 ml of the stock solution to 100 ml.

B. Preparation of Standard Curve

Aliquots of 0, 0.4, 0.8, 1.2, 1.6 and 2.0 ml of working TEP standard solution are accurately pipetted into screw-cap test tubes and water is carefully added to a total volume of 5 ml. Five millilitres of TBA reagent are added and the tubes tightly capped. After thorough mixing, the test tubes are heated in a vigorously boiling water bath for 45 minutes and cooled in tap water. Absorbance of the solutions is determined at 538 nm within one half hour of cooling, setting the blank (0.0 ml TEP) to zero.

A plot of absorbance versus concentration of TEP provides a standard curve (Figure 2) from which subsequent concentrations of TEP may be determined. The final concentrations of TEP in the above 10-ml volumes correspond to 0, 4.0, 8.0, 12.0, 16.0 and 20.0 (x 10^{-7}) moles per litre respectively.

C. Apparatus

The apparatus used consisted of a specific vertical distillation assembly (Figure 1A and 1B). Both distillation apparatuses can be used to produce identical TBARS values. However, the apparatus shown in Figure 1A uses less space and causes less operation errors. A Virtis 23 blender, a boiling water bath, a spectrophotometer (Bausch & Lomb spectronic 100), 15 x 125 mm screw-cap test tubes with teflon-lined caps, a 50-ml volumetric flask, and a 500-ml round bottom flask are used to fit the distillation assembly.

D. Recommended Procedure

(i) Fish Sample Preparation

Whole Atlantic mackerel *(Scomber scombrus)* and other fish samples from outside Halifax Harbour were used for this investigation. Fish was filleted, and the meat samples were homogenized in the food processor and stored at -40°C in 50-gm plastic containers.

(ii) TBARS Distillation

Pre-weighed 10-gm samples of finely chopped (Cuisinart food processor or blender) fish was placed in small containers and frozen immediately. Meat and skin samples were treated separately. Without thawing, a 10-gm portion of fish was transferred to the blender jar with 35 ml of distilled water and blended for two minutes or until the sample was finely divided. While blending, take a 500-ml round bottom flask to which have been added a few anti-bumping granules and 100 mg (approximately) each of propyl gallate and then the EDTA was prepared. The sample homogenate was transferred to the flask, and distilled water was added so that the total weight of the sample and water was 105 gm. The sample was flushed with nitrogen and 95 ml of 4N HCl was added. The distillation was started immediately and 50 ml of distillate was collected (volumetric flask). Thiobarbituric acid-reactive substance distillation should be completed within 35 minutes or less. The distillation rate was kept at about one to two drops per second.

The still, between samples, was rinsed with methanol and then distilled water. All joints must be tight and should be wet during distillation. Thiobarbituric acid-reactive substance distillates may be refrigerated overnight if necessary.

(iii) Spectrophotometric Determination

Pipet 5 ml of each TBARS distillate and 5 ml of TBA reagent into screw-cap test tubes, cover tightly and then treat as described in standard curve preparation. A blank of 5 ml of distilled water should be run simultaneously. Sample solutions with absorbance greater than 0.5 should be diluted with distilled water or alternately with the analyses being repeated using less TBARS distillate.

(iv) Calculation of TBARS Value

TBARS value is expressed as moles malonaldehyde per kilogram of fish. If a 5-ml aliquot of distillate obtained from 10 gm of fish is used, then TBARS value may be calculated from the simplified formula:

$$c \times 10^{7} = \text{TBARS} \quad (\mu\text{mole/kg fish}) \quad [1]$$

where c represents equivalent concentration in moles per litre of TEP determined from the standard curve. For aliquots other than 5 ml, the formula becomes:

$$\frac{5(\text{mls})}{\text{aliquot size (ml)}} \times c \times 10^{7} = \text{TBARS} \quad [2]$$

Example

In a run the following data was obtained:

Standard

Volume TEP_{5} std (1×10^{-5} M)	Final Concentration in Cuvette	Absorbance 538 nm
0	0	0
0.4 mls	0.4×10^{-6} M	0.077
0.8 mls	0.8×10^{-6} M	0.154
1.0 mls	1.0×10^{-6} M	0.193
1.2 mls	1.2×10^{-6} M	0.232
1.6 mls	1.6×10^{-6} M	0.309
2.0 mls	2.0×10^{-6} M	0.386

Mackerel Sample

Volume TBARS Distillate Used	Absorbance
3 mls	0.195
5 mls	0.325

Calculations

From the graph, an absorbance of 0.325 (for mackerel) corresponds to an equivalent TEP concentration of 1.68×10^{-6} M.

Therefore, from formula #1, TBARS concentration is:

$$1.68 \times 10^{-6} \times 10^{7} = 16.8 \mu\text{mole/kg fish}$$

Similarly, the 3-ml aliquot is calculated by formula #2:

$$5/3 \times 1.01 \times 10^{-6} \times 10^{7} = 16.8 \mu\text{mole/kg fish}$$

Concentration may also be derived from Beer's Law:

$A = \varepsilon bc$ where
A = absorbance
= extinction coefficient (slope of line in standard curve)
b = cuvette path length (usually 1 cm)
c = molar concentration TEP in cuvette

e.g. for absorbance of 0.325 using $\varepsilon = 1.93 \times 10^{5}$ as molar absorbability, TBARS value is calculated as:

$$c = A/\varepsilon b = \frac{.325}{1.93 \times 10^{5} \times 1} = 1.68 \times 10^{-6} \text{ molar}$$

RESULTS AND DISCUSSION

Comparison of Absorption Spectra

The absorption spectra from the distillate samples of fish tissue and TEP standards are compared in Figures 3A and 3B. Absorbance at 538 nm was chosen for the present TBARS determination to give a maximum sensitivity in the final spectrophotometric measurements. The changes of absorbances from 440 nm to 550 nm of various fractions of distillate were also plotted in Figure 3C. Some TBARS of the fraction IV and IX of distillate have second absorption peak at 500 nm which was also observed in our previous report (16). The spectra of other various carbonyls were also investigated as shown in Figure 4. Absorption at 500 and 450 nm (Figure 4) mainly from biomolecular oxidation reactions are important for some kinetic investigations (16, 25), but not useful for the fish quality assessment. Therefore, we use the first 50 ml of distillate for the recommended procedure even if the TBARS collection from the distillation is just 97% (Table 1). Those selected operation conditions for present rancidity evaluation give better reliable results.

Recovery From TEP Standard and Investigation on Interfering Substances

1,1,3,3 - Tetraethoxypropane (TEP) produces malonaldehyde (MA) and the standard TBARS curves for TEP standard of 0.0 to 2.0 μM as shown in Figure 2. A highly reproducible linear standard graph was obtained, and the molar absorbtivity of TEP ($1.90 \pm 0.03 \times 10^{5}$) is used to calculate the TBARS data. The recovery of TEP added to three fish fillet samples was also determined (Table 2). These data indicate that the recommended method gave fish quality analyses with a recovery error better than 5%.

Tests for interference from various biosubstances possibly present in fish tissue were carried out by adding about 10-50 mg of each compound to a 10-gm fish sample. All compounds, as listed in Table 3, did interfere slightly with the described TBARS method, giving a relative deviation of about 5% or less.

Application for Fish Quality Evaluation

By using the proposed procedure, TBARS values and relative standard deviations for various frozen and fresh fish fillets are presented in Table 4. The results indicate that this objective method can be satisfactorily applied for the evaluation of rancidity development in fish under various conditions and the TBARS shows with good reproducibility and sensitivity at the overall error of 7% from 10 gm of meat samples.

Thiobarbituric acid-reactive substances are the product of various reactions of oxidative rancidity from some polyunsaturated fatty compounds. Some kinetic studies under various frozen temperatures were reported earlier (15), and some autoxidations are still taking place when the fish is frozen at -40°C due to various proxidants and trace-transition metals present in fish tissues (6, 7, 14, 17, 24). The variations of TBARS value for tuna, mackerel, redfish held at +2, -15 and -30°C, respectively, have been plotted in Figure 5. The TBARS value changes in those fish that are well reproducible if some seasonal and sampling variations are eliminated. Therefore, TBARS value can be successfully employed as a quality indicator for the fish grading in terms of rancidity development.

It has been well established that the increases of peroxide value and free fatty acid content in fresh or frozen fish show good agreement with the TBARS values (5, 10, 15). Those relationships vary with temperature and other conditions (16, 29). Thiobarbituric acid-reactive substance value changes in fish samples exhibit good lineal relationships with the results from the organoleptic taste panels (1, 11, 14, 28, 41).

The correlation of TBARS value with taste panel data for herring, redfish, mackerel, cod and tuna have been reinvestigated and the recommended guidelines for assessing the rancidity development in fish are listed in Table 5. For objective quality evaluation, TBARS value less than 8 μmoles per kilogram of fish are indicative of excellent quality, while fish with TBARS value between 9 and 20 are acceptable. Fish flesh with TBARS value greater than 21 is unacceptable. These rancidity standards may require some adjustments for tuna since its TBARS formation is extremely rapid. Moreover, TBARS should not be used as the only parameter to evaluate fish quality; additional indicators, such as FFA, TVB, etc., should be used in order to have reliable quality assessments.

In conclusion, the described method for determining the TBARS values is a valuable tool for rancidity assessment as well as overall quality grading for both frozen and fresh fish.

REFERENCES

1. Anderson, K. and Danielson, C. E. 1961. Storage changes in frozen fish: A comparison of objective and subjective tests. Food Technol., 15: 55-56.

2. Asakawa, T., Nomura, Y. and Matsushita, S. 1975. On the reacting compounds in the TBA method for the determination of lipid oxidation. Research Institute for Food Science, Kyoto University (Uji).

3. Bernheim, F., Bernheim, M. L. G. and Wilbur, K. M. 1948. The reaction between thiobarbituric acid and the oxidation products of certain lipids. J. Biol. Chem. 174: 257-264.

4. Bishop, D. M. 1968. A critical examination of a TBA procedure for the measurement of malonaldehyde in fishery products. Fisheries Research Board of Canada. Halifax Laboratory. Halifax, Nova Scotia.

5. Botta, J. R. and Richards, J. F. 1973. Thiobarbituric acid value, total long-chain free fatty acids, and flavour of pacific halibut *(Hippoglossus stenolepis)* and chinook salmon *(Oncarhynchus tshawytscha)* frozen at sea. Can. J. Fish. Aquatic Sci., 30: 63-69.

6. Castell, C. H. and Bishop, D. M. 1969. Effect of hematin compounds on the development of rancidity in muscle of cod, flounder, scallops and lobster. Ibid., 26: 2299-2309.

7. Castell, C. H., Moore, B. and Neal, W. 1966. Cause and control of spurious thiobarbituric acid values from fish muscle in the presence of inorganic iron salts. Ibid., 23: 737-746.

8. Dahle, L. K., Hill, E. G. and Holman, R. T. 1962. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Archives of Biochemistry and Biophysics, 98: 253-261.

9. Deng, J. C. 1978. Effect of iced storage on free fatty acid production and lipid oxidation in mullet muscle. J. Food Sci., 43: 337-340.

10. Dyer, W. J. and Fraser, D. I. 1959. Proteins in fish muscle. 13. Lipid hydrolysis. Can. J. Fish. Aquatic Sci., 16: 43-52.

11. Greene, B. E. and Cumuze, T. H. 1981. Relationship between TBA numbers and inexperienced panelists' assessments of oxidized flavour in cooked beef. J. Food Sci., 47: 52-58.

12. Ho, S. Y. and Brown, W. D. 1966. Reactivities of lipids solvents with thiobarbituric acid. J. Food Sci., 31: 386-388.

13. Ke, P. J., Ackman, R. G. and Linke, B. A. 1975. Autoxidation of polyunsaturated fatty compounds in mackerel oil: formation of 2,4,7 - decatrienals. J. Amer. Oil Chem. Soc., 52: 349-353.

14. Ke, P. J., Ackman, R. G. and Nash, D. M. 1975. Proposed rancidity indexes for assessing the storage life of frozen mackerel. Fisheries and Oceans Canada, Halifax Laboratory, New Series Circular No. 52.

15. Ke, P. J., Ackman, R. G., Linke, B. A. and Nash, D. M. 1977. Differential lipid oxidation in various parts of frozen mackerel. J. Food Technol., 12: 37-47.

16. Ke, P. J. and Woyewoda, A. D. 1979. Micro determination of thiobarbituric acid value in marine lipids by a direct spectrophotometric method with a monophasic reaction system. Anal. Chem. Acta, 106: 279-284.

17. Ke, P. J., Nash, D. M. and Ackman, R. G. 1976. Quality preservation in frozen mackerel. Can. J. Food Sci. Technol., 9: 135-138.

18. Kohn, H. I. and Liversedge, N. 1944. On a new aerobic metabolite whose production by brain is inhibited by apomorphine, emetine, ergotamine, eqinephrine and menadione. J. Pharmacol., 82: 292-295.

19. Kwon, T. W. and Olcott, H. S. 1967. Thiobarbituric-acid-reactive substances from autoxidized or ultraviolet-irradiated unsaturated fatty esters and squalene. Institute of Marine Resources, University of California.

20. Kwon, T. W. and Watts, B. M. 1963. Determination of malonaldehyde by ultra-violet spectrophotometry. J. Food Research, 28: 627-630.

21. Kwon, T. W., Menzel, D. B. and Olcott, H. S. 1965. Reactivity of malonaldehyde with food constituents. J. Food Sci., 30: 808-813.

22. Kwon, T. W. and Olcott, H. S. 1966. Malonaldehyde from the autoxidation of methyl linolenate. Nature, 210: 214-215.

23. Lemon, D. W. 1975. An improved TBA test for rancidity. Fisheries and Oceans Canada, Halifax Laboratory, New Series Circular No. 51.

24. Maclean, J. and Castell, C. H. 1964. Rancidity in lean fish muscle. I. A proposed accelerated copper-catalyzed method for evaluating the tendency of fish muscle to become rancid. Can. J. Fish. Aquatic Sci. 21: 1345-1359.

25. Marcuse, R. and Johansson, L. 1973. Studies on the TBA test for rancidity grading. II. TBA reactivity of different aldehyde classes. J. Amer. Oil Chem. Soc., 50: 387-391.

26. Munkner, W. 1967. Application of the 2-thiobarbituric acid reaction (TBA-reaction) in fish technology. Translation series No. 943. Fisheries and Oceans Canada.

27. Placer, Z. A., Cushman, L. L. and Johnson, B. C. 1966. Estimation of product lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal. Biochem., 16: 359-364.

28. Ryan, B. A. and Stansby, M. E. 1959. Measurement of rancidity in fishery products by 2-thiobarbituric acid method. Commercial Fisheries Review, 21: 21-23.

29. Saslaw, L. D. and Waravdekar, V. S. 1965. Behavior of unsaturated fatty acids in the thiobarbituric acid test after radiolysis. Radiation research, 24: 375-389.

30. Saslaw, L. D., Corwin, L. M. and Waravdekar, V. S. 1966. Production of chromophoric substances in the thiobarbituric test. Arch. Biochem. and Biophys., 114: 61-66.

31. Schoenmakers, A. W. and Tarladgis, B. G. 1966. Reliability of the thiobarbituric acid test in the presence of inorganic iron. Nature, 504: 1153.

32. Shibata, N. and Kinumaki, T. 1979. An improvement of TBA procedure as the measure of the oxidative deterioration occurring in fish oils. I. Distillation procedure. Bull. Jap. Soc. Sci. Fish., 45: 499-503.

33. Shibata, N. and Kinumaki, T. 1979. An improvement of TBA procedure as the measure of the oxidative deterioration occurring in fish oils. II. Intact sample procedure. Bull. Jap. Soc. Sci. Fish., 45: 505-509.

34. Sinnhuber, R. O. and Yu, T. C. 1958. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. II. The quantitative determination of malonaldehyde. Food Technol., 12: 9-12.

35. Sinnhuber, R. O. and Yu, T. C. 1958. Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. Food Research, 23: 626-634.

36. Tarladgis, B. G., Watts, B. M. and Younathan, M. T. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. J. Amer. Oil Chem. Soc., 37: 44-48.

37. Tarladgis, B. G., Pearson, A. M. and Dugan, L. R. Jr. 1962. The chemistry of the 2-thiobarbituric acid test for the determination of oxidative rancidity in foods. I. Some important side reactions. Ibid., 39: 34-39.

38. Tarladgis, B. G., Pearson, A. M. and Dugan, L. R. Jr. 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Agric., 15: 602-607.

39. Vyncke, W. 1970. Direct determination of the thiobarbituric acid value in trichloroacetic acid extracts of fish as a measure of oxidative rancidity. Fette Seifen Austrichmittel, 72: 1084-1087.

40. Witte, V. C., Krause, G. F. and Bailey, M. E. 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. J. Food Sci., 35: 582-585.

41. Woyewoda, A. D. and Ke, P. J. 1979. Quality assessment of fatty fish by the 2-thiobarbituric acid distillation method. Fisheries and Oceans Canada, Halifax Laboratory, New Series Circular No. 63.

42. Yu, T. C. and Sinnhuber, R. O. 1964. Further observations on the 2-thiobarbituric acid method for the measurement of oxidative rancidity. J. Amer. Oil Chem. Soc., 41: 540-543.

43. Yu, T. C. and Sinnhuber, R. O. 1957. 2-Thiobarbituric acid method for the measurement of rancidity in fishery products. Food Technol., 11: 104-108.

TABLE 1 COMPLETION ON THE SEPARATION OF TBARS BY THE DISTILLATION METHOD

Fraction of TBARS Distillate	% Of TBARS In Distillate			
	TBARS From Fish		TEP Standard	
	This Fraction	Accumul. Fraction	This Fraction	Accumul. Fraction
Fraction I (0-10 ml)	29.8	29.8	29.6	29.6
Fraction II (10-20 ml)	24.2	54.0	24.0	53.6
Fraction III (20-30 ml)	19.1	73.1	19.5	73.1
Fraction IV (30-40 ml)	14.5	87.6	14.2	87.3
Fraction V (40-50 ml)	9.4	97.0	9.7	97.0
Fraction VI (50-60 ml)	2.6	99.6	2.3	99.3

TABLE 2 RECOVERY OF TEP STANDARD ADDED TO VARIOUS FISH SAMPLES BY USING THE RECOMMENDED METHOD

Fish Sample	TEP Added (μMole)	TBARS Value (μMole/Kg)	TEP Recovery (%)
None	0.040	4.0	100.0
	0.120	11.9	99.0
	0.250	25.5	102.0
Mackerel fillet A	None	2.8	N/A
	0.080	10.4	95.0
	0.150	18.5	104.0
Mackerel fillet B	None	28.5	N/A
	0.100	37.7	97.0
Herring fillet	None	10.6	N/A
	0.100	21.0	102.0
	0.300	38.6	95.0

TABLE 3 VARIOUS INTERFERENCES FROM SOME BIOCOMPOUNDS IN FISH TISSUE ON THE RECOMMENDED TBARS DETERMINATION

Compound	Amount Added (mg)	TBARS Value (μMoles/kg Fish)	% Of Variation
Control*	None	19.2	---------
Palmitic acid	5.1	18.7	- 2.6
Stearic acid	4.8	20.6	+ 7.3
Lecithin	52.0	19.7	+ 2.6
Cystine	52.3	19.9	+ 3.6
Cysteine	5.4	20.4	+ 6.2
Thiourea	5.2	19.3	+ 0.5
TMAO	47.1	18.5	- 3.6
TMA	5.2	17.8	- 7.3
DMA	5.1	18.3	- 4.7
Sorbitol	4.9	19.5	+ 1.6
Sucrose	50.8	18.9	- 1.6
Lactic acid	56.8	18.7	- 2.6
TBHQ	5.0	19.8	+ 3.1
TBHA	4.7	18.9	+ 1.4
$MnSO_4$	5.4	19.4	+ 1.0
$CuSO_4$	48.3	20.4	+ 6.3
Hemoglobin	4.8	19.6	+ 2.1
NaCl	50.4	18.8	- 2.1
$FeSO_4$	1.0	19.6	+ 2.1

* The samples of 10 gm of mackerel tissue were used as the control and all interference investigations.

TABLE 4 TBARS VALUE IN VARIOUS FRESH AND FROZEN FISH FILLETS DETERMINED BY THE DISTILLATION-PHOTOMETRIC METHOD

Sample	TBARS Value (μ Mole/kg Meat)	R.S.D.* (%)
Frozen Fillets		
Mackerel (skin on)	14.2	6.8
Mackerel (skin off)	5.6[2]	3.0
Herring	8.9[4]	5.2
Tuna	12.4	2.0
Tuna	28.6	4.1
Cod	11.3	5.0
Redfish	6.5[3]	4.5
Fresh Fillets		
Cod	2.4[1]	5.0
Mackerel A	3.6[5]	6.4
Mackerel B	25.4	1.0
Redfish	12.6	4.2

* Relative standard deviations were calculated from nine determinations.

TABLE 5 RECOMMENDED GUIDELINES ON THE RANCIDITY QUALITY ASSESSMENT FOR FRESH AND FROZEN FISH USING TBARS VALUES

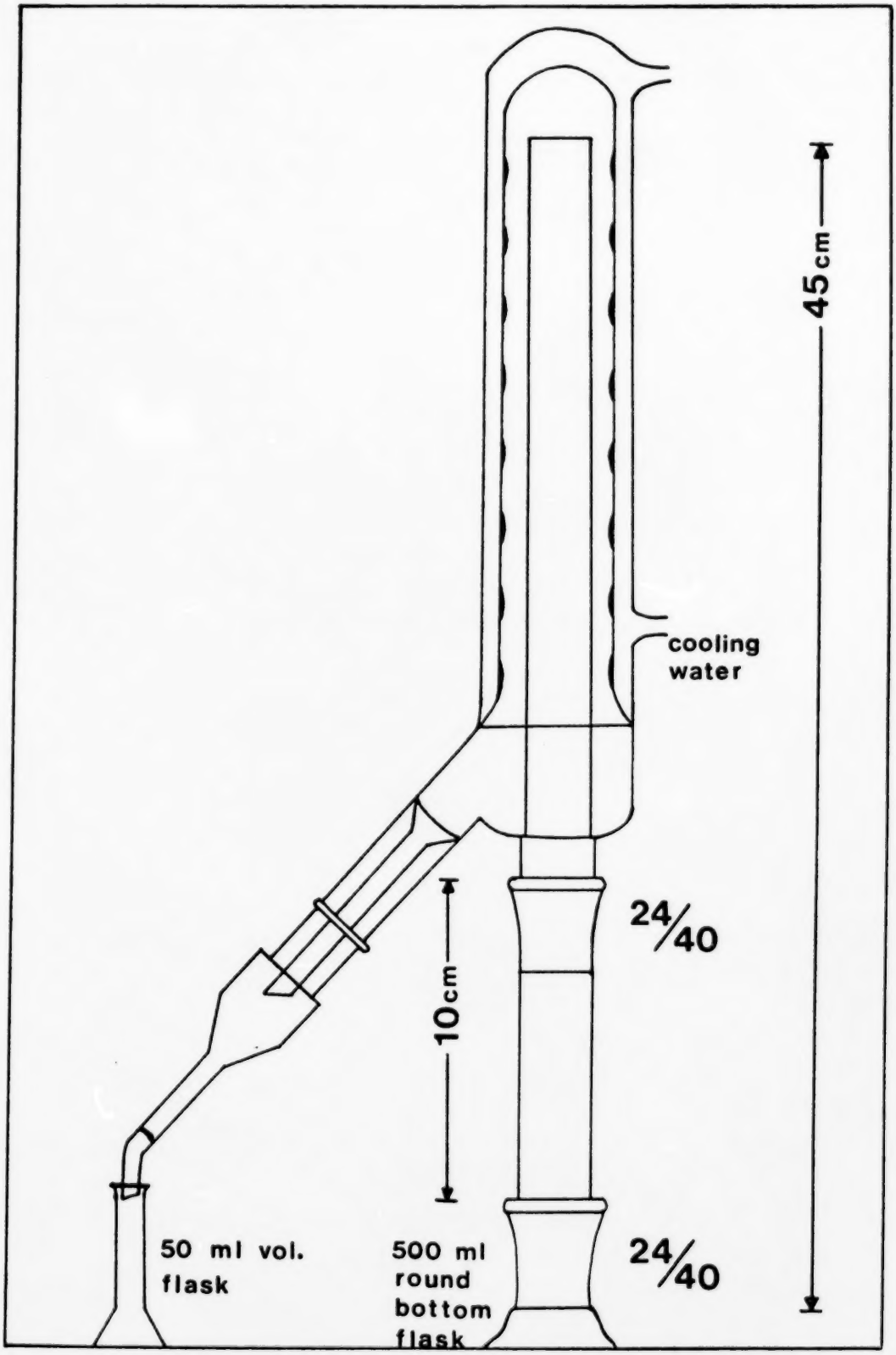
Degree of Rancidity	TBARS Value (μMoles/kg Fish)	Overall Quality *
Not rancid	0-8	Excellent
Slightly rancid	9-20	Good
Moderately rancid	Over 21	Unacceptable

* (1) For the objective quality evaluation, TBARS should be used with another quality parameter.

(2) The higher TBARS value may be needed for tuna grading.

FIGURE 1A - COMBINED DISTILLATION ASSEMBLY FOR TBARS ANALYSIS

FIG. 1A



TBARS DISTILLATION ASSEMBLY WITH CONDENSER-CONCENTRATOR

FIGURE 1B - DISTILLATION ASSEMBLY FOR TBARS ANALYSIS

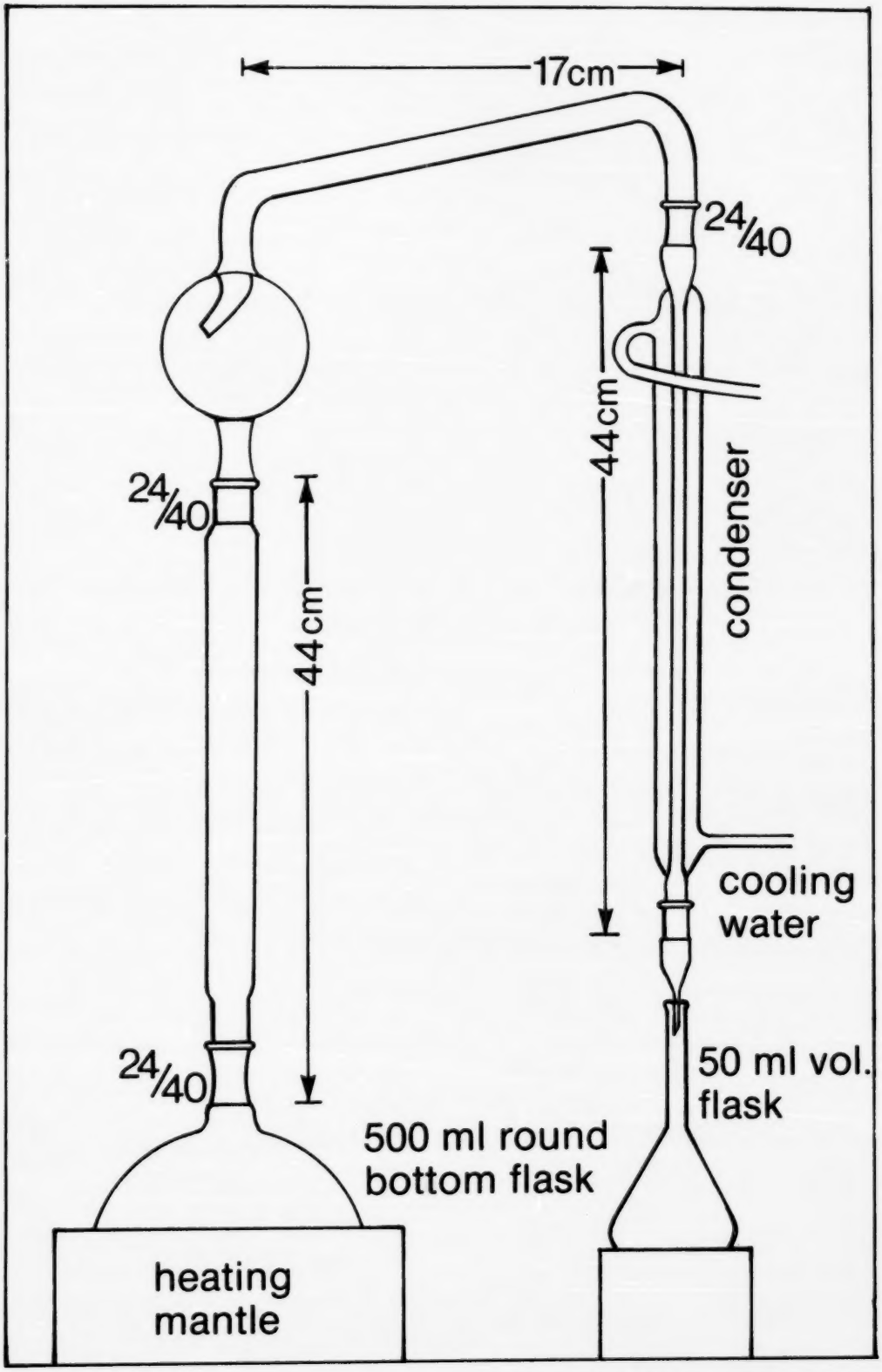


FIG. 1B

TBARS DISTILLATION ASSEMBLY

FIGURE 2 - STANDARD CURVE FOR TBARS DETERMINATION AT 538 nm
USING TEP AS THE STANDARD

FIG. 2

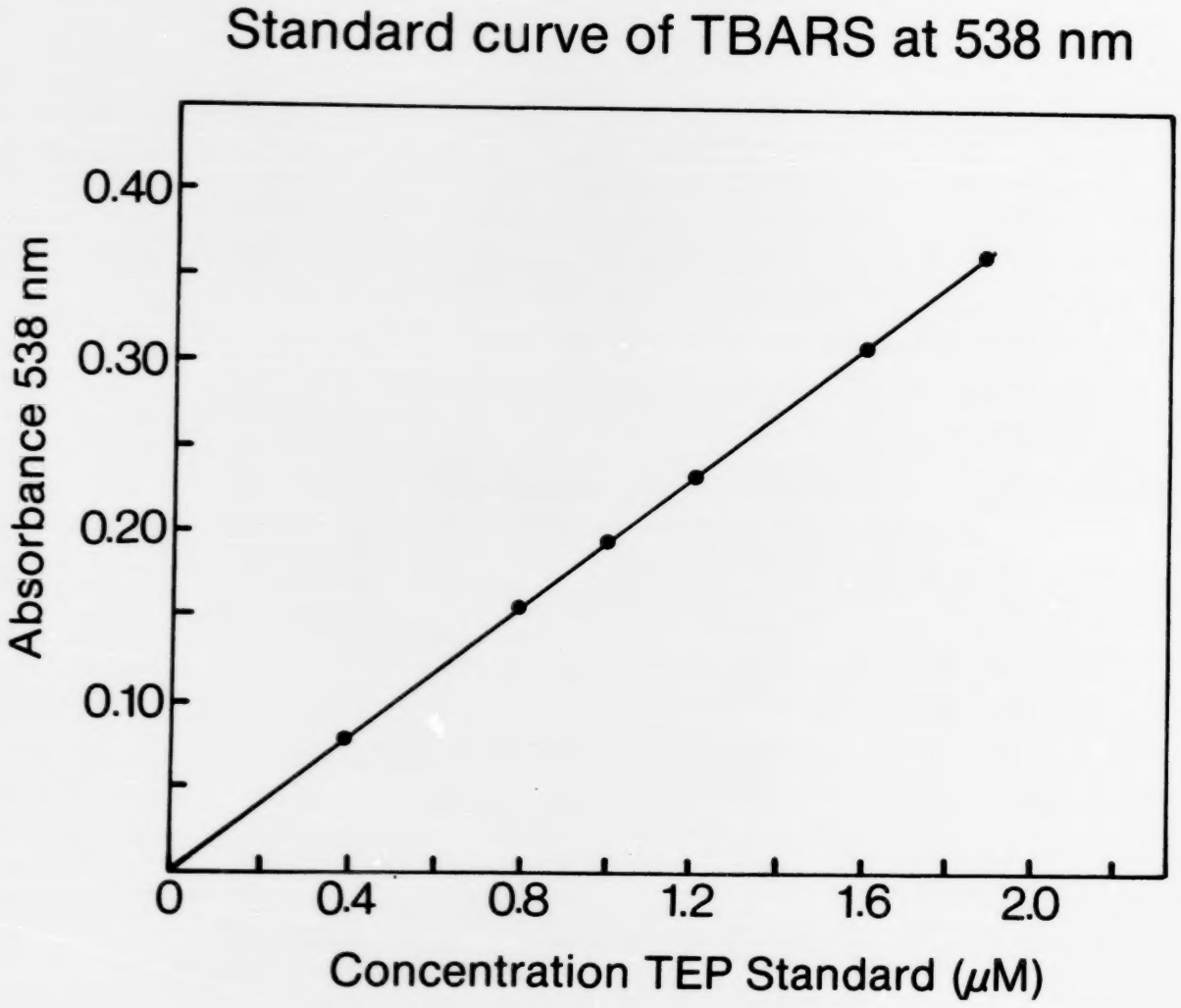

Standard curve of TBARS at 538 nm
Absorbance 538 nm
0.40
0.30
0.20
0.10
0
0.4
0.8
1.2
1.6
2.0
Concentration TEP Standard (μM)

FIGURE 3 - SPECTRA OF VARIOUS TBARS FROM FISH TISSUE

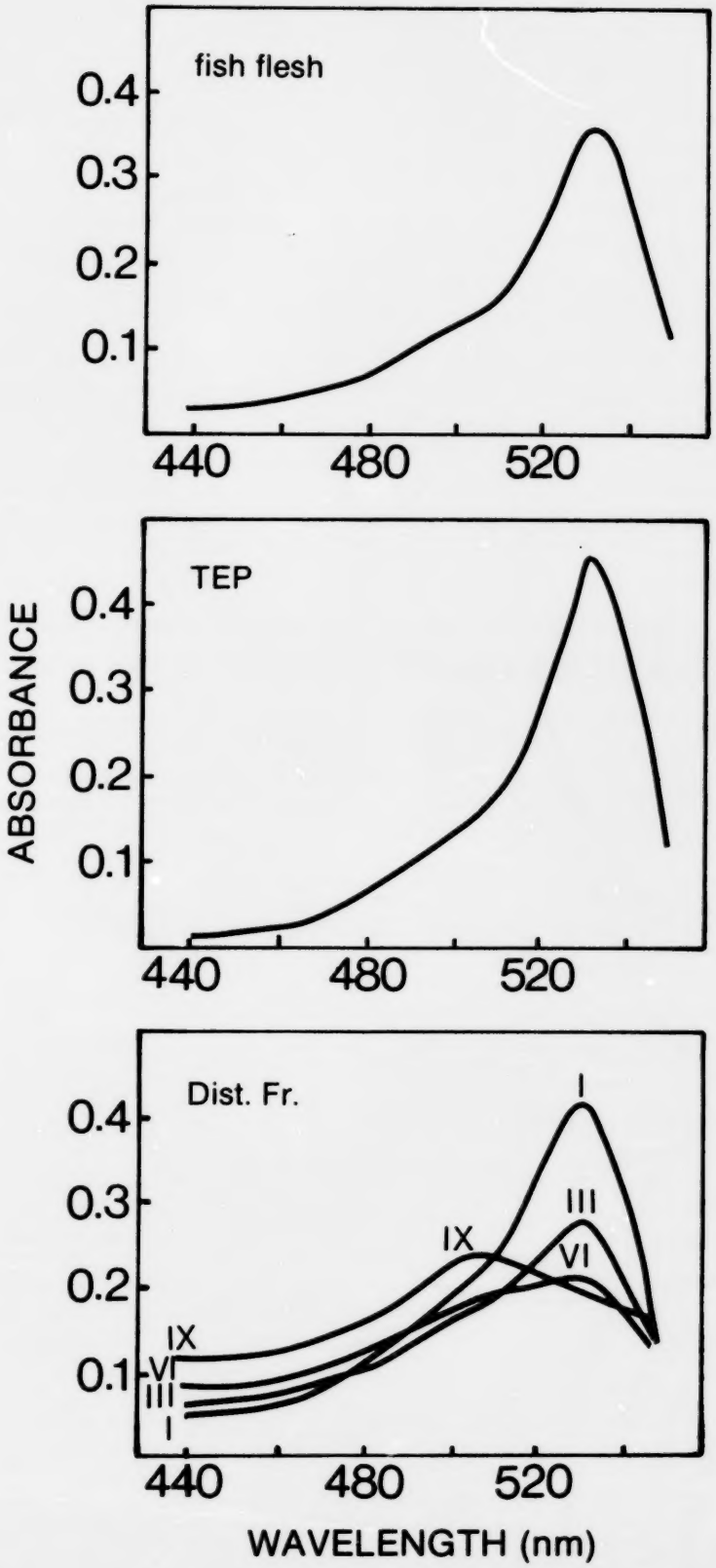

fish flesh
0.4
0.3
0.2
0.1
440
480
520
TEP
ABSORBANCE
Dist. Fr.
I
III
IX
VI
WAVELENGTH (nm)

FIG. 3

FIGURE 4 - ABSORPTION SPECTRA OF VARIOUS TBA-CARBONYL COMPLEX FORMED BY USING THE DESCRIBED DISTILLATION-PHOTOMETRIC OPERATION

FIG. 4

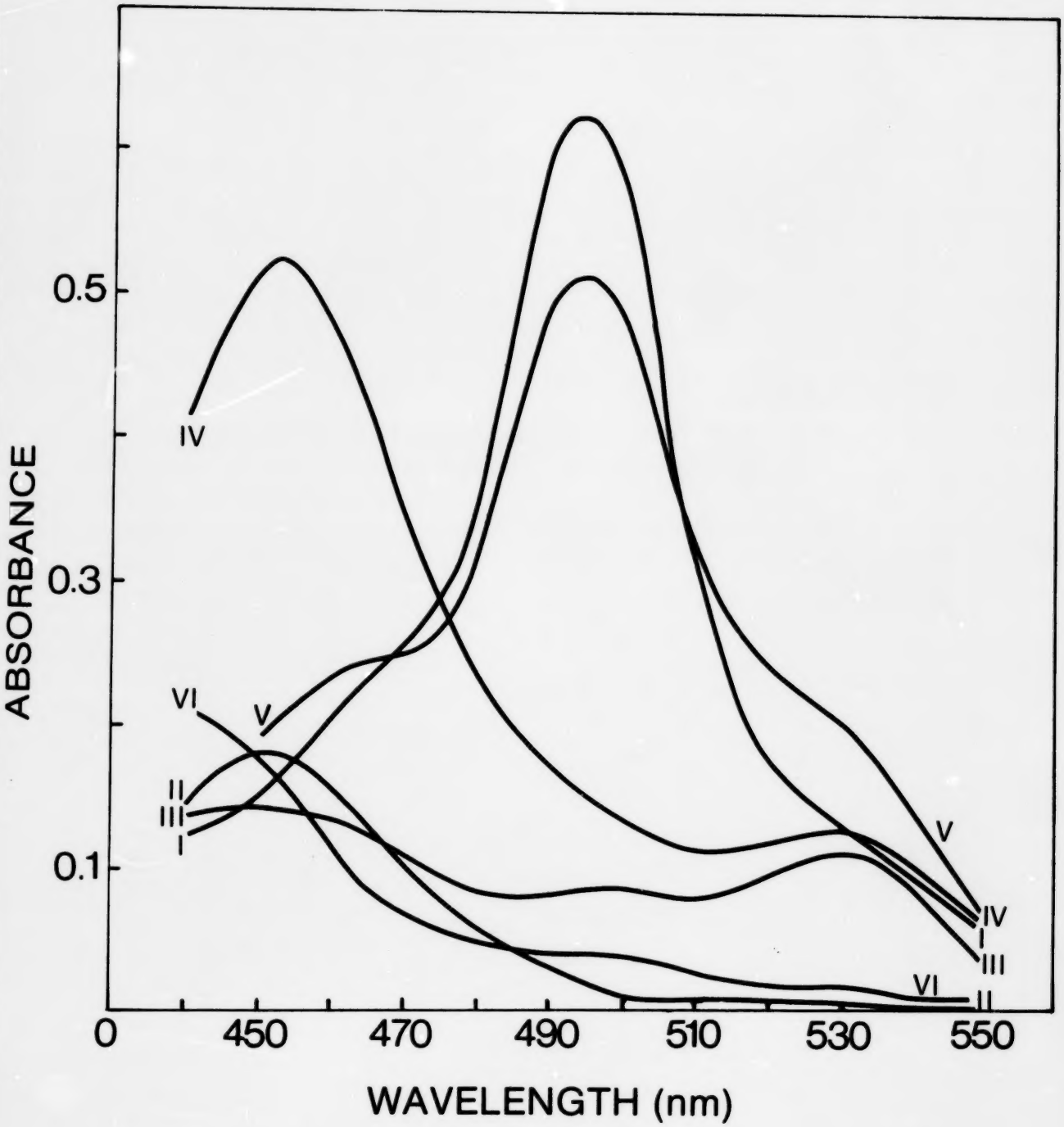
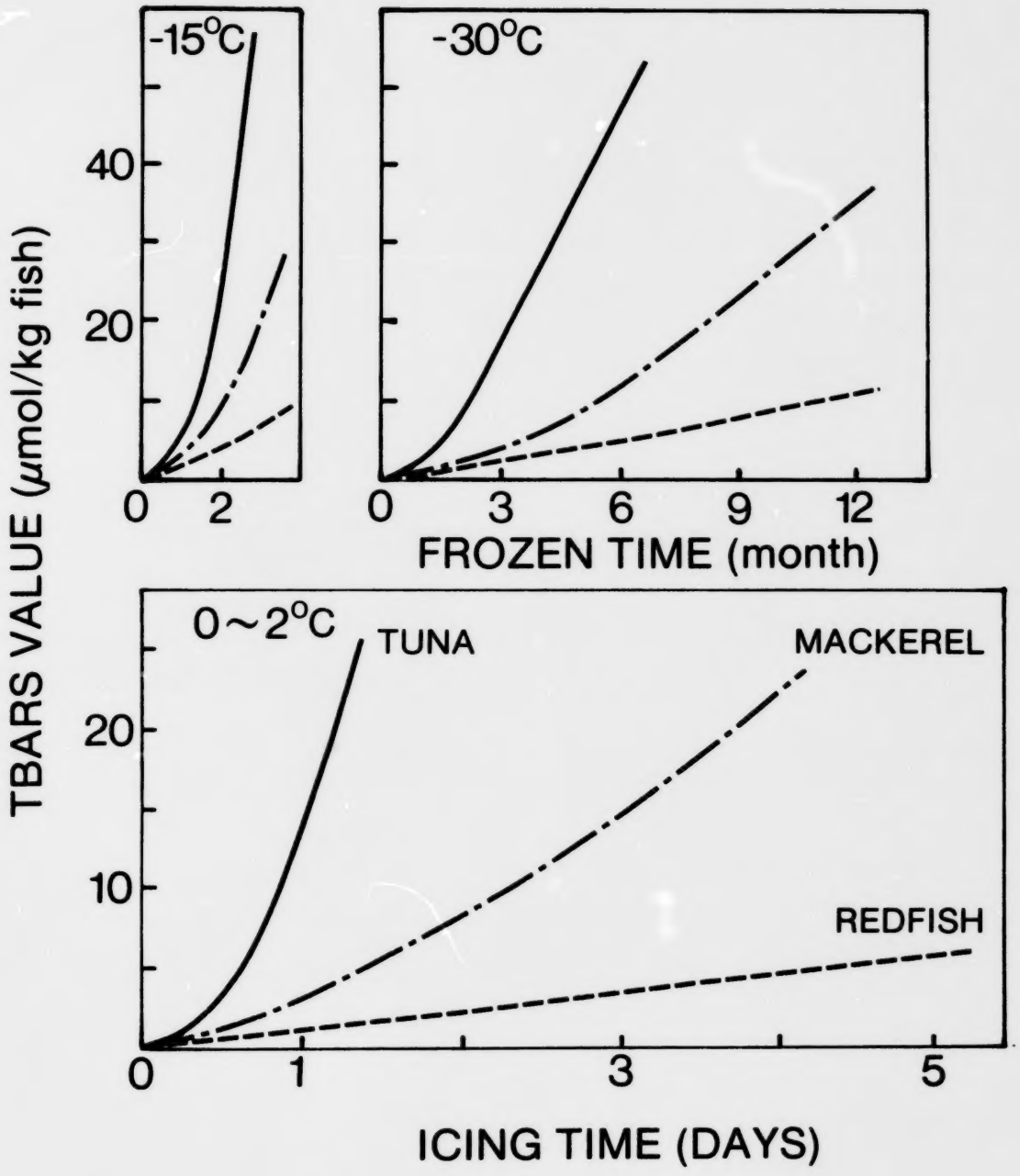

ABSORBANCE
0.5
0.3
0.1
0
450
470
490
510
530
550
WAVELENGTH (nm)
IV
VI
V
II
III
I
V
IV
I
III
VI
II

FIGURE 5 - TBARS VALUE CHANGE FOR VARIOUS FISH SAMPLES KEPT AT $-2^{o}C$, $-15^{o}C$ AND -30^{o} RESPECTIVELY

FIG. 5


-15°C
-30°C
40
20
0
2
0
3
6
9
12
FROZEN TIME (month)
TBARS VALUE (μmol/kg fish)
0~2°C
TUNA
MACKEREL
REDFISH
20
10
0
1
3
5
ICING TIME (DAYS)